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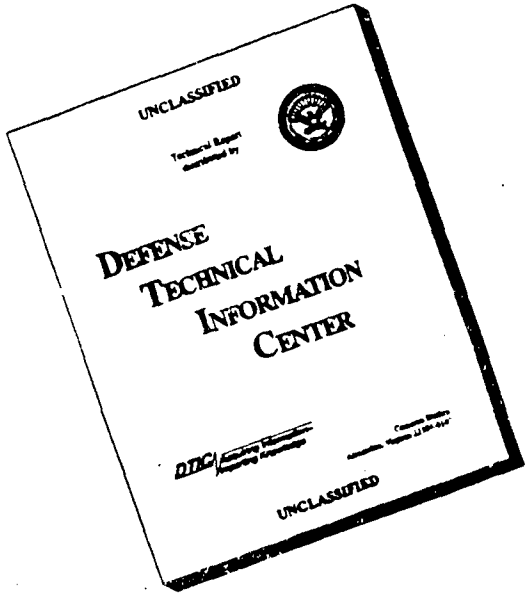

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EXPLORATORY DEVELOPMENT OF AN ULTRAFAST-CURING WOUND DRESSING

ANNUAL REPORT

MICHAEL SZYCHER
DONALD D. DEMPSEY

NOVEMBER 30, 1988

Supported by

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Thermedics Inc.
470 Wildwood Street
Woburn, Massachusetts 01888-1799

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We are developing a drug-dispensing field dermal dressing. The dermal dressing, which can be easily applied by an untrained person, contains a dual antibiotic to prevent bacterial infection.

The medicated dermal dressing is made of an ultra-fast-curing polyurethane oligomer which is designed to cure at room temperature and delivers drugs on a controlled, sustained and highly reproducible basis.

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SUMMARY

Thermedics Inc. is developing a second generation, controlled release dermal dressing. The self-adherent dressing, which can be easily applied, incorporates antimicrobial agents to aid in wound healing.

The new dermal dressing is a trilaminate composite. The air side of the trilaminate is a fabric impregnated with an aliphatic, medical grade polyurethane elastomer; the middle laminate is a controlled release layer, containing the microencapsulated medicinal agents, and the third laminate is a 1.0-mil-thick layer of acrylic based, pressure-sensitive adhesive.

The middle layer is fabricated from mixture of urethane and silicone oligomers, which are precompounded with medicinal agents, and is subsequently solidified (cured) upon mere exposure to low intensity UV radiation at room temperature. Solidification at room temperature is a vital consideration, because most drugs are rapidly inactivated upon mild heating. Once cured, the oligomer layer containing medicinal agents becomes a controlled release monolith, capable of dispensing drugs at a continuous and predictable rate.

FOREWORD

Future conflicts may have to be fought without the advantage of overwhelming American air supremacy. In the absence of air supremacy, it may not be possible to evacuate wounded American soldiers for proper medical treatment for at least several days. This situation implies that a wounded soldier would need to be treated in the field.

Based on this scenario, we embarked on the development of a new field dermal dressing. The new dressing would need to be applied without the benefit of prior medical training, during combat, and under all imaginable climatic conditions. The new dermal dressings incorporate antimicrobial agents to prevent or treat possible bacterial infection in soldiers with skin lesions. Currently available dressings do not meet these requirements.

Under research contract DAMD 17-88-C-8012, Thermedics is developing a second generation dermal dressing which speeds wound healing, incorporates pharmacologically active substances, and can be easily applied. This new dressing is based on an ultra-fast-curing liquid polyurethane oligomer. The oligomer can be easily precompounded with medicinal agents and, subsequently, cured in less than seconds at room temperature by illumination with UV radiation. Following cure, the dermal dressing delivers the drugs in a controlled, sustained release basis.

This second generation, medicated dermal dressing, when properly developed and tested, may become an ideal vehicle for the initial wound stabilization of wounded soldiers. Our research is being aimed at the development of a medicated dressing with the following characteristics:

- Oligomer cured at room temperature during manufacture; thus, even heat-sensitive drugs may be incorporated
- Dressing may be applied under any conceivable climatic condition by personnel.
- Dressing is highly compliant for physical comfort and is highly abrasion resistant, even when wet.
- Dressing is moisture permeable but does not permit penetration of water or bacteria.
- Dressing delivers antimicrobial agents in a controlled fashion.

I. RESEARCH OBJECTIVES

A. IDEAL REQUIREMENTS

The ideal second generation medicated field dressing should:

- Be soft and elastic, closely mimicking the mechanical properties of natural, intact skin.
- Display adequate adhesion to intact skin, but be minimally adhering to clot, so it may be removed at will.
- Control water vapor and oxygen exchange, thus maintaining a moist environment for rapid healing.
- Gradually deliver broad-spectrum antimicrobial agents that are nontoxic to the injured tissue.

These research objectives are aimed at the development of a field dermal dressing that provides immediate wound stabilization. This wound stabilization is expected to be accomplished through: (a) reduction of abrasion trauma, (b) easy removal without precipitating another bleeding episode, (c) promotion of normal wound healing under moist, aseptic environment, and (d) prevention of bacterial infection.

Incorporation of pharmocoactive agents is a key feature of the new dressing. The microcencapsulation of drugs into a polymeric matrix was made possible by the development of a room temperature, ultrafast UV curable liquid polyurethane oligomer. This is a crucial consideration, since most drugs are rapidly inactivated by mild heat. To ensure full pharmacological activity, the drugs should not be subjected to heat. This requirement was met by incorporating the drugs into the liquid matrix of the uncured oligomer followed by a room temperature, UV cure of the dressing.

Once cured, the dermal dressing, containing drugs, becomes a sustained release formulation. The dressing, in turn, once in contact with the wound and bodily fluids, provides immediate, direct, and controlled doses of drugs, targeted to the wound site, thus minimizing problems inherent in systemic drug delivery.

Promotion of the normal wound healing mechanism is another feature of the new field dressing. The dressing is semi-occlusive; i.e., it allows O2, CO2 and water vapor to permeate in physiological amounts, but it excludes bacteria. This feature is important because, under these conditions, the field dressing is capable of maintaining the wound moist, but aseptic. And, as explained in the following paragraphs, it is now apparent that moist, aseptic wounds heal faster.

B. HYPOTHESES

The above mentioned research objectives are based on the hypothesis that an elastic, semiocclusive dermal dressing, containing extended action pharmacological agents will provide soldiers with immediate wound stabilization. Our assumptions are that immediate wound stabilization will be accomplished through: (a) control of infection, and (b) promotion of normal wound healing mechanisms.

Therefore, our research goal is currently directed toward producing a "second generation" dressing capable of producing an aseptic microenvironment under the wound which is most conducive to rapid healing.

C. BACKGROUND

1. Wound Healing and Dressings

For centuries, the common understanding of wound healing remained relatively static. There was an awareness that an open wound was subject to the threat of infection. Optimal wound healing was thought to occur under a scab. Dressings were used to protect from bacterial invasion and infection. Dressing materials, traditionally composed of gauze, encouraged the drying of wounds to facilitate scab formation.

In the 1950's, observers realized that an unbroken blister healed more rapidly. Since the blister protects the wound surface with a layer of fluid, this realization led to a new understanding of wound healing (1).

Healing of partial-thickness damage has three major steps:

1. Epithelial Proliferation
2. Epithelial Migration
3. Dermal Proliferation

Complete epithelialization (steps 1 and 2) represents an effectively closed wound. The epidermal migration necessary to accomplish this closure is now understood to occur only over moist and healthy tissue.

Research in the 1960's, and published articles of the early 1970's, showed that the optimum conditions for steps 1 and 2 above (epithelialization) occurred under a dressing that maintained a moist environment. The development of the polyurethane products (a temporary artificial skin) arose from the recognition of this wound healing principle. The materials were utilized in the attempt to provide a moist environment much like nature's blister (2).

Prior to the studies on the potential effects of dressings on the repair process mentioned above, the medical community had thought that the surgical dressing mainly absorbed exudate, cushioned the wound site, and hid the site from the patient. This research illustrated that dressings can affect the response to the wound and even retard healing through dehydration or tissue damage during removal. It is now appreciated that dressings can serve to promote faster healing. Dressings can optimize epithelialization, reduce pain (which is associated with wound dehydration), and minimize local inflammation. If impregnated with drugs, they can also deliver medication.

Optimal wound healing occurs when the dressing material strikes a balance between dehydration and maceration (which results from accumulation of excess exudate). In addition to stimulating pain, dehydration leads to desiccation and cell death, undermining epithelial movement and wound closure. Prevention of dehydration can minimize eschar formation and inflammatory response. Maceration, which is stimulated by excess fluids and debris, is often accompanied by bacterial proliferation; it also has its own attendant negative effects on wound healing.

The desired balance between dehydration and maceration has had a direct impact on synthetic dressing development. The moist healing environment (to counter dehydration) has been the primary goal of materials development. Permeability to vapor (to counter maceration) has been a key element in the development of most of the materials. Some products have used other means to avoid maceration, such as special water-absorptive properties. As experience with the synthetic dressing materials and their applications grows, the clues for next generation product improvements to further the support of the sound healing process are stimulating new avenues of research.

2. Clinically Available Dressings

At the present time, the most successful dressing for split-skin graft donor sites and burn care is Op-Site. Op-Site is Smith and Nephew's trade name for a polyether-based, polyurethane, moisture vapor permeable membrane compounded with silica gel; the polyurethane membrane is hydrophillic, and is coated at the edges with a polyvinyl ethyl ether adhesive (3).

This polyurethane moisture vapor permeable membrane has been shown to offer significant advantages over conventional dressings in the management of superficial injuries, split-skin graft donor sites, and burn sites. The hydrophilic polyurethane dressing protects wounds from bacterial contamination, while providing a suitable environment for rapid wound healing (4); patients seem more comfortable with the polyurethane dressing than with standard dressings (5); and rapid healing is produced by enhancement of re-epithelialization through increased mitotic division and migration of epidermal cells (6).

In spite of these impressive credentials, Op-Site, as a field dressing is impractical for two important reasons. First, the application of Op-Site often requires two trained people and sometimes three (7); a successful military field dressing should have the capability of being applied by only one person. Second, Op-Site is a semi-occlusive dressing only, devoid of pharmacological agents, that may aid in healing of the skin lesion.

Tegaderm, a 3M product that entered the market in 1981, is the second ranked transparent, polyurethane, membrane dressing. Tegaderm's contribution to the market and its competitive advantage is found in its packaging and in its application configuration. The product is packaged sterile in a water resistant packet between two paper liners. One liner releases, while the other serves as a frame and a window to ease application.

The value of Tegaderm, emphasized by 3M, is in IV care. Company literature focusing on IV applications highlights three sizes ranging from 5 cm x 7 cm (2 in x 2-3/4 in) to 15 cm x 20 cm (6 in x 8 in). With Tegaderm, as with Op-Site, the major complaints revolve around the difficulty of application, especially the difficulty of removal; both of these products adhere tenaciously to both intact skin and the scab. During removal, not only is the patient subjected to acute localized pain, but the wound site frequently starts rebleeding when the scab is forcibly disturbed.

3. Thermedics Medicated Dermal Dressing

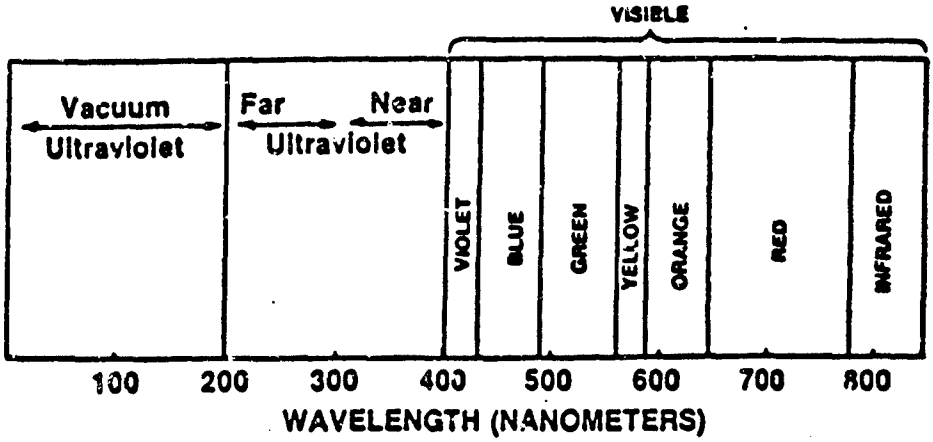
We propose to use a self-adherent medicated field dressing capable of being applied by one person to the skin site. The new field dressing can be applied by one person since it will be provided as a thin, semi-occlusive membrane, capable of being easily draped over complicated body contours.

Dermal dressings designed to prevent bacterial infection will be composed of a semi-occlusive outer layer of Tecoflex medical-grade polyurethane. The middle layer will be composed of our exclusive, patented UV-curable Tecoflex polyurethane oligomer, encapsulating antimicrobial agents for topical administration to wounds. This middle layer will be loaded with either: a) gentamicin sulfate and clindamycin, b) chlorhexidine, or c) bacitracin, neomycin and polymixin B sulfate. Finally, there will be an inner layer of hypoallergenic acrylic pressure-sensitive adhesive for easy application to the periphery of the wound.

A common concern in clinical use of UV-curable materials is that there are repeated reports of toxicity, arising from the use of bone cements, such as polymethylmethacrylate, and also from the use of UV visible light curable dental materials. We have concluded, from our review of the literature, that toxicity is traceable to the presence of leachable, unreacted monomers, following apparent "cure" of the materials. Unreacted monomers, in contrast to the polymer, are low molecular weight cytotoxic fluids, capable of migration and leaching into surrounding tissue.

UV visible light curable dental materials depend on photochemical dissociation of a photoinitiator to produce free radicals. Either UV or visible light may be used to cleave the photoinitiator and thus initiate the polymerization reaction. Ultraviolet radiation is defined as that portion of the electromagnetic spectrum between 200 and 400 nm, and visible light is defined as that portion of the electromagnetic spectrum between 400 and 780 nm, as shown in Figure 1.

Figure 1. Electromagnetic Spectrum



In the clinical use of radiation curable composite restorative materials, two related factors are of concern: (1) the rate and extent of the polymerization reaction, and (2) subacute toxicity to surrounding tissues. Radiation curable composite restorative materials were initially screened for overt toxic effects; subcutaneous, intramuscular and intraosseous tests showed that implanted materials do not trigger inflammatory or allergic responses (8, 9).

Despite these favorable results in animal tests, the gradual exfoliation of clinical implants after five or more years raises the possibility that subacute toxicity may manifest itself after prolonged periods in situ. In order to avoid toxicity, our UV curable dermal dressing will only utilize medical-grade polyurethane composed of pre-reacted monomers. Both Tecoflex UV and Tecoflex TS are synthesized fully from low molecular weight reactants in our laboratories.

4. Battlefield Dermal Dressings

We have developed a family of ultrafast-curing urethane/silicone oligomers which, when exposed to UV radiation, produce elastomeric films capable of serving as semi-occlusive field wound dressings. The films have oxygen, carbon dioxide and water vapor permeabilities resembling those of intact skin. In addition, these oligomers may be precompounded with pharmocologic agents, thus behaving as extended-action, controlled release formulations.

This new family of biomedical-grade oligomers, when properly

developed and tested y become an ideal military field wound dressing for t.. ..ollowing reasons:

- Oligomers are synthesized from nonexotic, off-the-shelf chemicals; thus, the dressing will be inexpensive.
- All the necessary manufacturing technology is present at Thermedics; therefore production lead time will be minimal.
- The field wound dressing can be applied by nonmedical personnel.
- The dressing is highly compliant for physical comfort.
- The dressing will help achieve infection control and will promote normal wound healing.

Infection control (from pathogenic bacteria and opportunistic invaders), will be accomplished by incorporation of antimicrobial agents. Promotion of normal wound healing mechanisms will be accomplished by the use of an abrasion-resistant, field-curable polymeric membrane, which is: (a) noninflammatory and nonantigenic to the wound, (b) compliant as skin, (c) similar to skin in oxygen permeability, and (d) similar to skin in water vapor transmission characteristics.

Incorporation of pharmacological agents is a key feature of the new wound dressing. The microencapsulation of drugs into a polymeric matrix was made possible by the development of a room temperature, ultrafast, UV curable polyurethane oligomer. This is a crucial consideration, since most drugs are rapidly inactivated by mild heat. To insure full pharmacological activity, the drugs should not be subjected to heat. This requirement was met by incorporating the drugs into the liquid matrix of the uncured oligomer followed by a room temperature, UV cure of the dressing.

Once cured, the wound dressing, containing drugs, becomes a sustained release formulation. The dressing, in turn, once in contact with the wound and body fluids, provides immediate, direct, and controlled doses of drugs, targeted to the wound site, thus minimizing problems inherent in systemic drug delivery.

This unique combination of properties makes our dermal dressings an innovative solution for skin injuries by promoting healing, minimizing bacterial infection, and maintaining a clean wound under combat conditions. Development of this family of dressings should decrease morbidity from wound infections incurred during combat and reduce lost duty time. Our laboratories and biomedical staff stand ready to work with the USMRDC to bring this exciting concept to clinical fruition.

5. SELECTION OF ANTIMICROBIALS

The selection of topical antimicrobials for use in battlefield dressings is quite different from the selection process involved in conventional systemic therapy. The ideal topical antimicrobial agent should:

- Be poorly absorbed through skin for maximum kill potential at the applied site
- Be bactericidal at low local concentrations
- Have as broad a spectrum as possible
- Be mutually compatible with other antimicrobial agents, and complimentary in spectrum

Quantitative in vivo test procedures to determine the efficacy of topical antimicrobial agents were performed by Leyden and co-workers at the University of Pennsylvania (10). This comprehensive study ranked the efficacy of the most frequently used antimicrobial agents based on five tests: occlusion, expanded flora, persistence, ecological shift and serum inactivation. Results from these studies suggest that clindamycin and gentamicin are effective and complementary antimicrobial agents for use as topical agents against a variety of micro-organisms likely to be encountered in the battlefield environment.

Our survey of available antimicrobials suitable for incorporation into medicated wound dressing yields the following summary presented in Table 1.

TABLE 1
Summary of Best Antimicrobial Topical Agents

AGENT	CHARACTERISTICS	EFFECTIVE AGAINST	INEFFECTIVE AGAINST
Gentamicin	Aminoglycoside. Broad spectrum. Available as 0.1% topical ointment. Absorbed transdermally.	Gram ⊖ aerobic (i.e., e.coli enterococci) proteus, klebsiella	Anaerobes (i.e., clostridia, bacteroides fragilis)
Clindamycin	Similar to gentamicin. Not transdermally absorbed, thus potentially better topical agent.	staphylococci, streptococci, clostridia, bacteroides	e. coli, pseudomonas aeroginosa
Neomycin	Aminoglycoside. Broad spectrum. Poorly absorbed transdermally. Used 0.25 - 0.5% topically.	Gram ⊖ staphylococcus aureus	Streptococcus pyogenes
Polymyxin B	Cyclic polypeptide, elaborated by bacillus polymyxin. Available as topical ointment in conjunction with other agents.	Gram ⊖ bacilli pseudomonas aeroginosa	Proteus, serratia. Inactivated by serum.
Bacitracin	Polypeptide, isolated from bacillus subtilis. Available in topical forms. Narrow spectrum. Inactivated by water.	Gram ⊕ staph aureus streptococcus pyogenes	Bacterial resistance develops slowly. Oral administration develops super infections and GI upsets.
Chlorhexidine	Aqueous solution containing 19 to 21% chlorhexidine gluconate	Gram ⊕ and ⊖ bacteria/pseudomonas	

Based on these considerations, we have been developing a dual-loaded medicated battlefield wound dressing. The dual-loaded dressing contains gentamicin and clindamycin, since these two agents in combination should effectively control those bacterial infections most likely to be encountered during battlefield conditions.

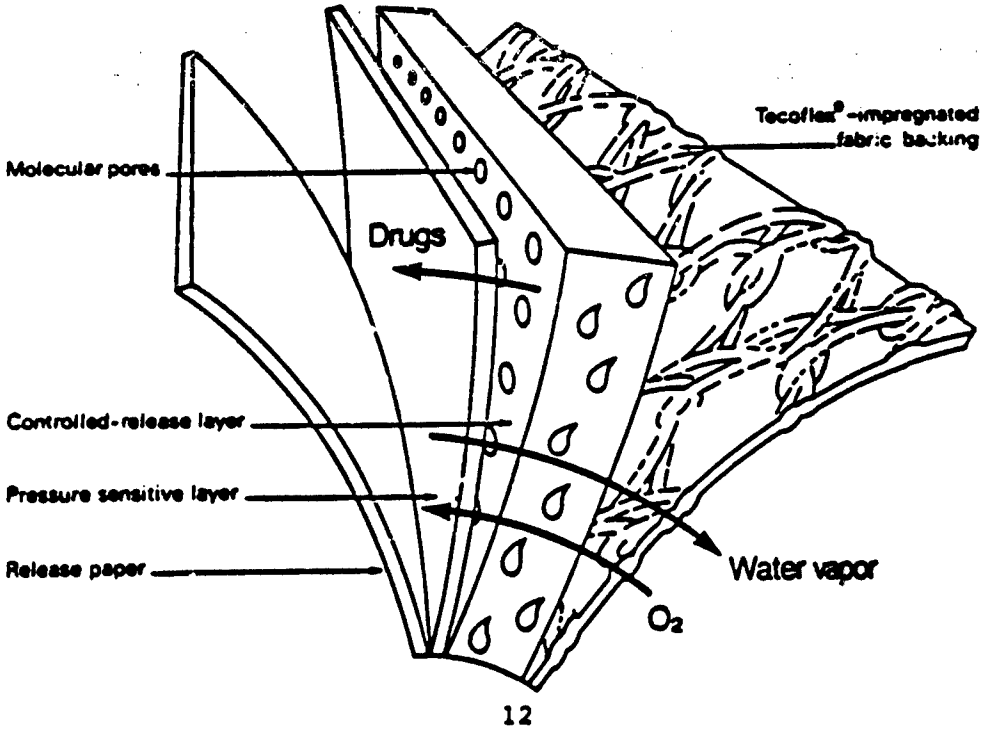
II. WORK TO DATE

Our dermal dressing is a trilaminate composite, shown in Figure 2. The air side of the trilaminate is a polyurethane impregnated fabric. The middle laminate is a controlled release layer containing the microencapsulated medicinal agents; and the third laminate is a 1.0 mil thick layer of acrylic based, pressure sensitive adhesive. The entire trilaminate composite is attached to release paper; prior to use, the soldier removes the release paper, and the dressing is applied to the wound. The dressing is held onto intact skin by means of the pressure sensitive adhesive.

The fabric was specifically selected for its ability to stretch like skin. Intact, healthy skin is anisotropic; that is, it stretches more in one direction than in another. The fabric mimics this property and, as a result, the new wound dressing is very comfortable once applied, because it "gives" like skin. In addition, incorporation of the fabric into the dressing imparts drapability previously unattainable by commercially available thin film dressings, since it does not wrinkle when the bandage is removed from the release paper.

FIGURE 2

The Thermedics Antimicrobial Dermal Dressing, Containing Gentamicin Sulfate in the Controlled-Release Area



However, the most important technical breakthrough of the new dermal dressing is our development of non-toxic, tissue compatible oligomers which cure under UV radiation. Curing by UV radiation is a breakthrough in medical-grade polymer technology, since it allows ultra-fast curing (solidification) of biocompatible materials in a matter of seconds at room temperature.

Utilization of UV curing oligomers permits the production of ingenious drug dispensing dermal dressings. The liquid oligomer may be compounded with pharmacological agents, yet it will solidify upon mere exposure to low intensity UV radiation at room temperature. Solidification at room temperature is a vital consideration, because most drugs are rapidly inactivated upon mild heating. Once cured, the oligomer containing drug(s) becomes a controlled-release monolith, capable of dispensing drugs at a continuous and predictable rate.

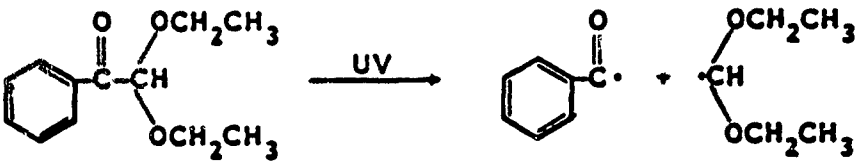
In our technology we utilize a physical mixture of vinyl-terminated polyurethane oligomer and a low molecular weight silicone fluid. The silicone fluid acts as a fugitive excipient, which exudes to the surface, reducing the tendency of clots from adhering to the dressing.

A. SYNTHESIS OF VINYL-TERMINATED POLYURETHANE OLIGOMERS

The polyurethane oligomer comprises a diisocyanate, a macroglycol, and an acrylyl chain terminator which provides the necessary vinyl end groups. Incorporation of a photoinitiator into this oligomer, and subsequently UV bombardment, results in a flexible, elastomeric and highly abrasion-resistant cured film. This film is expected to result in a superior field dermal dressing when extended-action therapeutic agents are compounded into the oligomer.

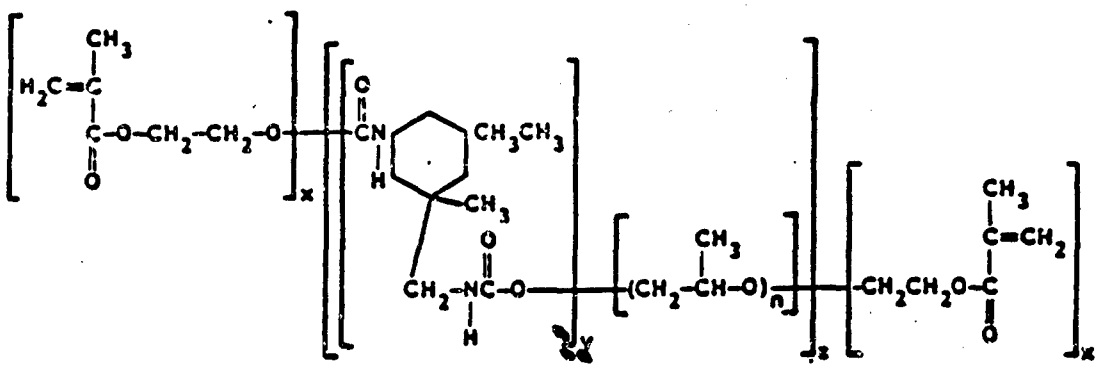
Specifically, we have produced UV curable polyurethane films, with high mechanical properties, such as 1500 psi ultimate tensile strength, 300 percent ultimate elongation, and excellent abrasion resistance by reacting 25.4 percent by weight of isophorone diisocyanate with 57.2 percent of 1000 Dalton polypropylene glycol (PPG). This isocyanate terminated prepolymer is chain extended with 13.3 percent by weight of hydroxyethyl acrylate. The final product, designated as an oligomer (shown in Figure 3) was then further compounded with 3.8 percent by weight of diacetoxyacetoben-phenone (the photoinitiator). The photoinitiator is activated under UV illumination to produce two free radicals, as shown in Figure 4. Polymer curing proceeds at room temperature when the free radicals generated by the photoinitiator react with the vinyl end groups, resulting in additional polymerization.

FIGURE 3
UV-Induced Free Radical Formation



BENZOPHENONE INITIATOR

FIGURE 4
Acrylic-Terminated Polyurethane Oligomer



The above constituents were emulsified and cast unto 250 mil thick films and exposed for 60 seconds to UV radiation from a commercially available UV source to produce the above mentioned mechanical properties.

During the first year, we synthesized a variety of urethane oligomers to maximize those properties most desirable in a field dressing, such as tensile strength and hardness. In our trials, we need only vary the molecular weight of the PPG to reduce the experimental matrix.

In varying the molecular weight of the PPG, we were guided by well-known principles in polymer chemistry. These principles state that as the molecular weight of the PPG decreases, the tensile strength and hardness decrease concomitantly. Inversely, as the molecular weight of the PPG increases, the tensile strength and hardess increase, thus allowing us to tailor the properties of the finished, cured film.

B. COMPOUNDING

The polyurethane oligomer, photoinitiator, PEG 200 and silicone oil are intimately mixed at 60°C to produce a thorough dispersion of the liquids. Once adequate dispersion of the liquids has been achieved, and the photoinitiator has been stabilized at 80°C, gentamicin and clindamycin powders are added slowly and ground into the mixture by means of a mortar and pestle.

The mixture is next applied in the form of a 250 um thick membrane onto the Tecoflex saturated fabric. The oligomer layer is cured by illumination from UV curing lamps, emitting a radiation spectrum covering the range from 320 to 440 um. The curing is performed in continuous ovens, under a polyethylene cover to protect the uncured membrane from oxygen, moisture, or other airborne contaminants.

C. PRESSURE-SENSITIVE ADHESIVE

To apply the dressing, and to keep it in place for the desired time period, a pressure-sensitive adhesive must be used. Delivery of drugs by a medicated dressing utilizing pressure-sensitive adhesive to maintain effective wound contact demands the marriage of three unrelated disciplines: pharmaceutical technology, polymer technology, and pressure-sensitive adhesive technology.

In our development of the field dressing, we have been guided by a number of key principles. These principles are encompassed in pharmaceutical, polymer, and adhesive technologies, and will be discussed in the following order: (1) adhesion to skin, (2) cohesive strength, (3) anchorage of adhesives, (4) skin irritation, (5) drug-oligomer-adhesive interaction, and (6) shelf stability.

1. Adhesion to Skin

Skin adhesion is a fundamental property required to hold any device in place. However, adhesive properties should be such that the dressing can be removed after the required residence time in an unremarkable manner. Skin adhesion should be balanced between: (a) the adhesion level required for secure holding regardless of patient movement, perspiration level or bathing, and (b) ease of removal when dosage is complete.

The most desirable adhesive system will also show uniform adhesion to skin versus time with only moderate adhesion build-up or loss. Also, the range of values observed should be statistically reproducible and as small as possible.

Adhesion level to the patient dosage site with a dermal dressing should only be enough to effectively keep the device in place for the necessary dosage period. Higher levels of skin adhesion should be avoided where possible, since high skin adhesion levels increase the incidence of excoriation during removal. Higher than necessary levels of skin adhesion also increases the probability of skin sensitization and irritation with repeated use on the same site. In our medicated dressing, we have chosen an acrylic-based, pressure-sensitive adhesive that builds adhesive to the skin site rapidly, plateaus, and thereafter maintains uniform adhesion for up to seven days. Upon removal, we have observed a minimum of adhesive residue left on the skin site, and removal has been unremarkable.

2. Cohesive Strength

This is the ability of the adhesive to stay together and to stay in place under load, i.e., resist shear. Good cohesive strength is also vital for clean removal from the skin with minimum residue. It is a manifestation of the visco-elastic properties of a particular system.

Cohesive strength, or lack thereof, is a function of the molecular weight and molecular weight distribution. Addition of relatively low molecular weight tackifying agents to compounded adhesives affects the molecular weight distribution. Adhesive processing during coating can also directly influence final molecular weight distribution.

Positive tests for good cohesive strength in vivo are the unit staying in place on the patient (not sliding) and unremarkable removal with no visible adhesive residue left on the skin.

In our wound dressing, we selected a pressure-sensitive adhesive which displayed sufficient cohesive (or internal) strength to remain in place, yet it peeled from the skin cleanly. Cohesive strength was not adversely affected by either ethylene oxide or gamma radiation sterilization, and was unaffected by temperatures between 95°F and -20°F.

3. Anchorage of Adhesive

The pressure-sensitive adhesive, which is designed to hold a medicated wound dressing to a soldier's skin, must on the obverse side stay adhered to the dressing. Keeping the adhesive firmly attached to the dressing is referred to as adhesive mass anchorage.

Adhesive mass anchorage is most easily tested in a direct manner. The tests are essentially qualitative - you either have it or you don't. An effective test that can be done without instrumentation is to simply fold the adhesive film composite pressure-sensitive side upon itself and press to-gether to ensure good contact. Then peel one end back on itself creating a 180 degree peel test.

Acceptable mass anchorage is also demonstrated by lack of adhesive transfer from one surface to the other. Still another sign of good mass anchorage is a uniform adhesive appearance after adhesive separation by the above tests. Unacceptable mass anchorage is gross transfer or delamination of adhesive from its support film or layer. Mass adhesive anchorage is critical to device performance. First, loss of mass anchorage may cause the device to fall off (in the worst case). Secondly, poor mass anchorage with attendant separation of device layers can cause dosage interruption, and/or dose dumping. In our medicated wound dressing, all components (saturated fabric, oligomer layer and pressure-sensitive adhesive) were carefully chosen for compatibility with the foreseen field service demands.

4. Skin Irritation

It is an unnatural condition for the skin to be covered with an adherent wound dressing, and to keep potential skin irritation, we have considered the following parameters:

- Skin irritation potential (rubber, silicone or acrylic)
- Length of time worn
- Drug adhesive interaction
- Adhesive permeability/porosity

In terms of skin irritation potential, rubber-band adhesives have shown the greatest potential; silicones were excellent, but changed tackiness after gamma sterilization; acrylics offered the best combination of properties.

Acrylics were shown to remain stable in contact with human skin for about seven days, and no drug/adhesive interaction was observed. Acrylics were also the most permeable/porous adhesives tested; this is important, since the more permeable/porous an adhesive is, the more it allows skin to breathe or respire resulting in less tendency toward skin irritation.

This is particularly important in a field wound dressing that may

not be replaced for several days. Under these conditions, large patches of skin will be continually covered by an adhesive dressing, which may lead to skin maceration. The selected acrylic adhesive has demonstrated the lowest tendency toward skin maceration, since it is highly porous.

5. Drug/Adhesive Interactions

The possibility of drug/adhesive interaction is an important consideration as it may change:

- Drug potency as a function of time
- Device wear characteristics
- Skin adhesion
- Skin irritation

Drug/adhesive interaction can affect skin adhesion. This can manifest itself as a softening of the adhesive mass making it too tacky with loss of cohesive strength. It may also cause irritation due to high skin adhesion. Excessively high drug levels can also work in the opposite direction and dry up the mass with resultant loss of tack or quick-stick.

We are cognizant of skin irritation phenomena which can result from unforeseen drug/adhesive interactions. To date, our tests have shown that drugs maintain their integrity and pharmacological activity when incorporated into our medicated wound dressing system.

6. Shelf Stability

The best designed system is of little value if its performance is lacking when it is finally used. Substandard shelf stability may be manifested by incorrect dose delivery or deterioration of adhesive characteristics.

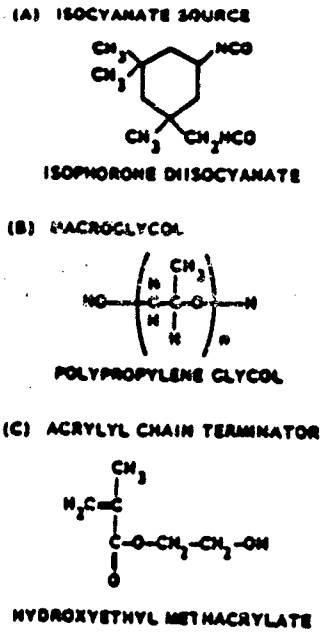
The choice of a pressure-sensitive adhesive polymer system will play a major role in shelf stability of the adhesive component of the delivery device. While there are probably exceptions, it has generally been demonstrated that synthetic rubber/natural rubber resin adhesives deteriorate most quickly with time. Acrylic polymer pressure sensitives show exceptional aging properties, and have thus become our choice for use in the manufacture of medicated field wound dressing.

III. PROGRAM STATUS

A. TASK 1. Make Quality Controlled Vinyl-Terminated Urethane Oligomer

The vinyl-terminated urethane oligomer is composed of three reactants: isophorone diisocyanate, polypropylene glycol and hydroxyethyl methacrylate, as shown below in Figure 5:

FIGURE 5
Monomers Used in the Synthesis of UV Curable Oligomer



The oligomer is prepared under continuous nitrogen purge; the isocyanate (24.8%) and glycol (58.75%) are preweighed and added to the reactor under agitation, while slowly raising the temperature to 60°C. At this time, one half of the catalyst (Cotin 430, M & T Corp.) is added, and the reaction maintained for three hours.

At this time, the hydroxyethylmethacrylate (16.45%), and the remainder of the catalyst are charged to the reactor, and the mixture is allowed to exotherm to a maximum of 110°C. The oligomer is allowed to cool to 90°C for an additional two hours, at which time the oligomer is considered synthesized.

To verify the completeness of the synthesis, a sample of the oligomer is run in a spectrophotometer. If free NCO appears at 2240 nm, small aliquots of methacrylate are added to scavenge any unreacted isocyanate; the absence of free isocyanate is confirmed with additional IR scans.

The finished oligomer is filtered, and packaged in plastic containers. The properties of the oligomer are: viscosity at 2 °C = 370 cps, color = clear to slightly pale yellow. The finished oligomer is now considered ready for the addition of the solid photoinitiator. The solid photoinitiator is solubilized into the oligomer by heating at 60°C, until all the solid has been solubilized. This can be confirmed visually.

We have produced enough urethane oligomer to prepare all necessary wound dressings used during year 1, utilizing the suppliers in Table 2.

TABLE 2
Raw Materials Suppliers

Ingredient	Supplier	Weight %
Isophorone Diisocyanate (IPDI)	Thorsen Chemical	24.8
Polypropylene Glycol (PPG 1025)	Union Carbide	58.75
Hydroxy Ethyl Methacrylate (HEMA)	Rohm & Haas	16.45

B. **TASK 2.** Make Quality Controlled Vinyl-Terminated Silicone Oligomer

During trials at USAIDR, it was observed that wound dressings tended to adhere to dried blood clots. The adhered blood clots would start rebleeding when the dressings are removed from the wounds.

USAIDR requested the development of a dressing that would not permit adherence to blood clots. Our staff began a search for a suitable way to prevent adhesion; we concluded that polyurethanes (being relatively tacky) could not be made non-adherent.

Silicone gums, on the other hand, were known to be non-adherent to biological tissue. We synthesized a series of UV curable silicone gums, which exhibited some lubricity. However, the physical properties of these UV polymerized elastomers were very poor, with low tear resistance, thereby rendering them unsuitable as dermal dressings.

In the course of our research, we discovered that a low viscosity, medical-grade silicone oil, designated as DC0200 by Dow Corning Corporation, when incorporated into the uncured polyurethane oligomer would eventually migrate to the surface, and thereby prevent biological adhesion.

This liquid silicone oligomer remains mobile within the polyurethane matrix, forming a unimolecular layer at the dressing interface. This unimolecular layer (which is replenishable from within the matrix) prevents biological adhesion, since clots cannot adhere to silicone oil.

We have incorporated 2%, 3%, 4%, and 5% by weight of silicone oligomer into the urethane oligomer, and fabricated dressings for in vivo evaluation.

C. **TASK 3.** Optimize and Determine Adhesive Characteristics and Gas Transmission Rate

1. Optimize Adhesive Characteristics of Dressings by Varying Concentrations of Urethane and Silicone Oligomers

We provided USAIDR with a total of 30 individually packaged, presterilized island dressings. The 2" x 2" dermal dressings,

with a 1" x 1" island were:

(a) 6 dressings, containing 2% by weight silicone (1.32 mg/dressing)
(b) 6 dressings, containing 3% by weight silicone (1.98 mg/dressing)
(c) 6 dressings, containing 4% by weight silicone (2.64 mg/dressing)
(d) 6 dressings, containing 5% by weight silicone (3.30 mg/dressing)
(e) 6 dressings, with no silicone (controls)

2. Determine the O2/CO2 Transmission Rate

It is widely accepted that dermal dressings need to be "breathable", to prevent tissue maceration and excoriation. The term "breathable" refers to the ability of the dressing to allow transmission (i.e., diffusion) of oxygen into the wound, and carbon dioxide gas into the air. This is considered important, since oxygen tends to inhibit the proliferation of anaerobic bacteria; conversely, the carbon dioxide gas produced by cellular metabolism needs to be cleansed.

Therefore, it is important to fabricate dermal dressings that are not only antibacterial, but are capable of "breathing" as well. This is a difficult requirement, since most film forming polymers do not "breathe." For example, well known films, such as polyester, polyetheylene, vinylidene chloride, and acrylonitrine butadnene sytrene (ABS), display the following transmission rates at a one mil film thickness: (see Table 3)

TABLE 3

Permeation Rates of Selected Polymers

Film Former	Gas Transmission (1 mil film) CO2	O2
Polyester	15 - 25	3.0 - 5.0
Vinylidene Chloride	3 - 5	.42 - .8
Polyethylene	718 - 4700	185 - 985
ABS	150 - 200	50 - 70

Units (cc/100 in2 24 hr. atm.)

For this reason, polyesters are used in packaging of potato chips to prevent loss of taste, odor, crispness, etc. Vinylidene chloride and polyethylene films are widely used in supermarkets to wrap meat and maintain redness and freshness by excluding oxygen. ABS displays a high CO2/O2, making it a potential candidate for use in "breathable" dressings, but it cannot be used in medical applications because of the unacceptable high level of acrylonitrile monomer (a suspected carcinogen).

Fortunately, polyurethanes are among the most "breathable" of film-forming polymers. Generally, when high breathability with high strength is necessary, the polyurethane elastomers are selected. Therefore, we expected that our Spandra dressings, which are totally based on polyurethane elastomers, would have good "breathability" even throughout the entire composite, allowing oxygen to reach the wounded tissue while permitting CO2 to diffuse into the atmosphere.

We estimated the steady state rate of O2/CO2 transmission through Spandra, in accordance with ASTM D-1434-32 method. This measurement provides quantitative estimates for the permeability of single pure gases through films and composites.

The samples were tested for gas transmission rate following Procedure M which is the manometric method. The specimens were conditioned overnight under a vacuum of 0.2 mmHg on one side, and the test gas on the other side. The rate of pressure in the test cell was measured using a mercury manometer; from this pressure change the Gas Transmission Rate was calculated.

The samples are mounted in a gas transmission cell so as to form a sealed semibarrier between the two chambers. One chamber contains the test gas at high pressure, and the lower chamber, at a vacuum, receives the permeating gas. In Procedure M, the lower pressure chamber is evacuated, and the transmission of the gas through the samples is indicated by an increase in pressure, as shown in Figures 6 and 7.

FIGURE 6
Manometric Gas Transmission Cell

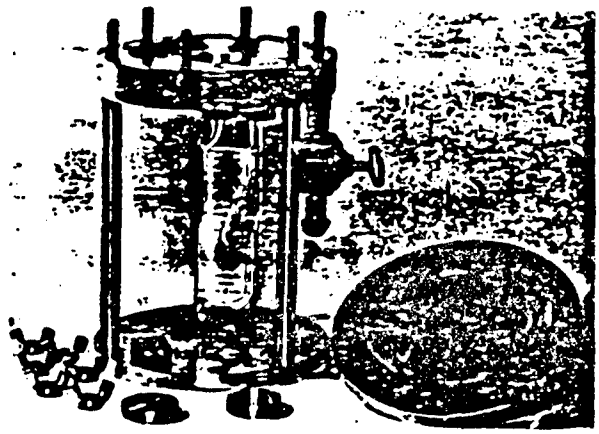
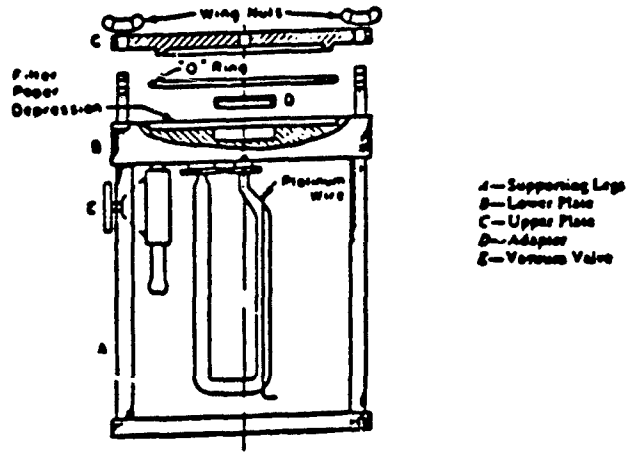


FIGURE 7
Schematic View of Gas Transmission Cell



The permeance P, in SI units are calculated from the following relationship:

$$P = g(h)V/(t - t_0)$$

where:

$$g(h) = -\frac{1}{ART}\left[\{V_1 + a(2\rho_0 + h_0 - h_L)\} \cdot \ln\left\{1 - \frac{(h_0 - h)}{P_0 - (h_L - h_L)}\right\} + 2a(h_0 - h)\right]$$

a = area of capillary $\overline{AB}$, mm^2.
A = area of transmission, cm^2.
h_0 = height of mercury in the capillary leg at the start of the actual transmission run, after steady-state conditions have been attained, mm.
h = height of mercury in cell capillary leg at any given time, mm.
h_B = maximum height of mercury in the cell manometer leg from the datum plane to upper calibration line B, mm.
h_L = height of mercury in cell reservoir leg from datum plane to top of mercury meniscus, mm.
P_0 = upstream pressure of gas to be transmitted,
R = universal gas constant 8.3143×10^3 L·Pa/(mol·K),
t_0 = time at the start of the actual transmission run, h, after steady-state conditions have been attained.
t = time, h.
T = absolute temperature, K,
V_{BC} = volume from B to C, µL,
V_{CD} = void volume of depression, µL, and
V_1 = $(V_{BC} + V_{CD})$, µL.

The values obtained for standard Spandra (fabric polyurethane alone), Island Dressing (2% and 5% silicone) are given below:

TABLE 4
Results of Gas Permeation Rates for Spandra Wound Dressings

Sample Identification	Test Results (CC/100 in2 24 hr. atm) CO2	O2
Standard Spandra	4200	1730
Island Dressing 2% Silicone	114	19
Island Dressing 5% Silicone	124	23

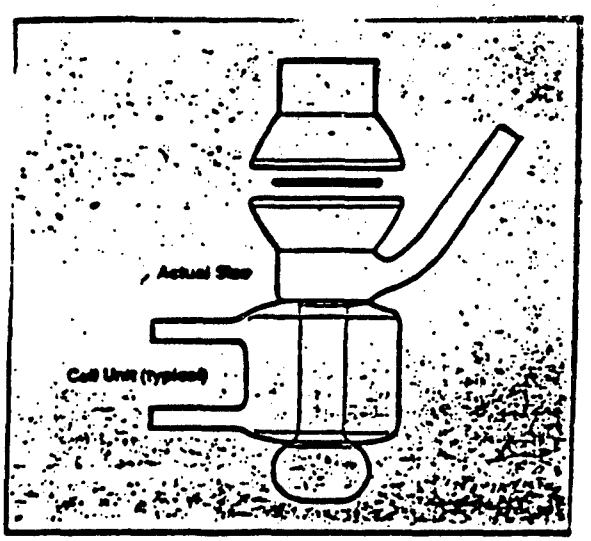
This data proves that the Spandra dermal dressing is "breathable" to O2/CO2 throughout the polymer matrix composite. This argues well for the overall performance of this antimicrobial wound dressing.

D. TASK 4. Make Prototype Dressings Impregnated With Gentamicin and Clindamycin

The release kinetics of gentamicin and clindamycin from the Spandra dressing would guide selection of the optimal dermal dressing. The selection criteria is dependent on the known Minimum Inhibiting Concentration (MIC) of gentamicin and clindamycin. The goal is to exceed the MIC for both drugs for a period of three days.

In vitro release kinetics have become an established means of predicting the diffusion of drugs from medicated films. In the late 1970's, Dr. Thomas J. Franz at the University of Washington, developed and commercialized a diffusion cell to evaluate the steady-state release kinetics into a infinite sink. The Franz cell is shown diagrammatically here:

FIGURE 8
Schematic View of Frantz Cell



As shown, the Franz cell consists of a water jacketed, magnetically stirred chamber with an O-ring joint and cap for fastening the medicated dressing. During operation, the cell is filled with distilled water (receptor solution) and the wound dressing is attached so that the active, medicated side is bathed by the water. The test is started, and the receptor is withdrawn at predetermined intervals, and assayed for the eluted concentration of the drug. The temperature is maintained constant, with continuous agitation of the receptor fluid, insuring reliability and reproducibility of data.

We developed an HPLC assay method for the determination of clindamycin and gentamicin using retractive index and electrochemical detectors respectively. The standard calibration curves were constructed for both antibiotics from the HPLC assay procedure.

1. HPLC Determination of Clindamycin

A rapid and reproducible high pressure liquid chromatographic determination of clindamycin was employed to quantitate the elution rates of various concentrations of clindamycin phosphate from Spandra dressings. The method utilizes reverse phase paired ion chromatography in conjunction with an ultrasensitive Waters 410 Refractive Index Detector.

Existing refractive index detectors have been used for the detections of aminoglycosides and more recently for the analysis of clindamycin. However, existing refractive index detectors can only be used to detect relatively high aminoglycoside concentrations, typically in the 25-50 mg/ml range. We required detection limits in the 25-50 mcg/ml range.

The difficulties inherent in the low level detection of aminoglycosides have forced investigators to seek other methods. The most common method in the literature requires the aminoglycoside to be derivatized with a fluorescent compound, such as flourescamine. Unfortunately, derivatization is well known to be a cumbersome, difficult to reproduce procedure, which greatly complicates the analysis.

We found that throughout the use of a Water's 410 Ultrasensitive Refractive Index Detector, the difficult derivatization step was eliminated while maintaining high levels of detection in the required 25-50 mcg/ml range.

Two prototype 6 mil films were prepared using an aggressive Spandra adhesive as substrate. Each of these films contained a

predetermined level of clindamycin phosphate loading as calculated by the clindamycin lot potency analysis (790 mcg/mg). The two levels of clindamycin per one square inch patch per 6 mils of thickness were chosen as follows:

16.6 mg/in2/6 mil thickness
20.0 mg/in2/6 mil thickness

These films were conditioned five days at room temperature prior to testing. Three samples of each film were punched using a 1.400 inch diameter die. These discs were placed in the Franz dissolution apparatus, described as follows:

> The dissolution apparatus was composed of separate 20 ml Franz cells containing a self-stirring mechanism. All cells were connected to the same constant temperature bath (37°C) by means of a manifold water system and allowed to equilibrate to 37°C. At that temperature, the cells were assembled with the test films in contact with the preheated water. One half milliliter aliquot was removed through the test port using an insulin syringe. The half milliliter extraction was replaced in the test cell with an equal amount (0.5ml) HPLC grade water. Each of the test aliquots were diluted with 0.75ml of a methanol diluent yielding a solutions of clindamycin phosphate dissolved in the HPLC mobile phase. Standard concentrations of clindamycin phosphate solutions were prepared in the mobile phase. The six standard concentrations in micrograms per milliliter were as follows: 25, 50, 100, 200, 300 and 400.

Our procedure utilized the following Waters Association apparatus: a 590 programmable solvent delivery module, a Nova Pak C-18 column (3.9 mm x 30 cm), a U6K injector system capable of delivering 200 microliters of sample, Model 410 refractive index (R.I.) detector exhibiting ten times the resolution of a 401 R.I. detector. All data were collected on a M730 data module and recorded as percent of area. The mobile phase was composed of methanol-water (HPLC) Grade 60:40), glacial acetic acid (0.035M) and pentane sulfonic acid sodium salt (0.005M), at an apparent pH of 4.4.

The mobile phase flow rate was 0.75 ml/mm at 3000 psi. The mobile phase was prepared as a theoretical four liter batch with all ingredients except the water. One liter of the methanol concentrate was removed and used to dilute the ex tracted test aliquots. The remaining three liters of concentrate was diluted to 5 liters of mobile phase with water, filtered and deaerated. The

same lot of mobile phase was used for standards and test aliquots.

Based on this innovative technique, we were able to generate the elution kinetic curve shown in Figure 9. The figure shows two curves, corresponding to a 20 mg/patch loading, and a lower curve corresponding to a 16 mg/patch loading. As expected, the 20 mg patch elutes clindamycin faster than the 16 mg patch.

2. HPLC Determination of Gentamicin

Gentamicin is an effective aminoglycoside antibiotic used in the treatment of serious Gram negative bacterial infections. Like other aminoglycosidic chemotherapeutic agents, gentamicin has a narrow therapeutic range. Consequently, a reliable and fast method of analysis is critical. Microbiological (11) enzymatic (12), hemagglutination inhibition (13) and radioimmunoassays (14) have been developed. Also, several methods for the analysis of this drug in serum (15) and plasma (16) have been reported.

However, these methods are tedious, time consuming or require the derivatization of the drug with chromophoric moieties to permit UV or fluorescence detection. The method reported here uses simpler chromatographic conditions, requires no derivatization and is also linear. Size exclusion chromatography or Gel Permeation Chromatography (GPC) was chosen for two reasons: the resolution of gentamicin sulfate into its isomers was not necessary; the separation of gentamicin from body serum was not required. These reasons combined with the high solubility of gentamicin in water made an aqueous mobile phase and hydrophilic GPC column practical. Electrochemical detection was chosen due to the nature of the drug molecule. The electrochemical detector relies upon the electroactive amino groups present in the drug molecule. These amino acid groups can be oxidized and the resulting current is proportional to the amount of the drug present.

Pure USP grade distilled water was filtered through a 0.22 um membrane filter and used as the mobile phase. Gentamicin sulfate powder containing 625 mcg/mg active was received from Chemwerth, and used as is. The wound dressings were made at Thermedics and 25 mm discs were cut out and used for the diffusion studies. The samples were extracted using Franz diffusion cells.

Chromatography was performed on a Waters Associates uPorasil 60°A 3 x 30 cm column, at a flow rate of 1 ml/min., using a Waters Solvent delivery module #570 and a Waters U6K injector. The detector used was an ESA Coulochem Model 5100A fitted with a Model 5010 Standard Analytical Cell, and the data recorded using a Waters Data Module M730 integrator.

Various concentrations, ranging from 25 mcg/ml to 400 mcg/ml of gentamicin sulfate were prepared in filtered distilled water and used to prepare a calibration curve. Ten microliters each of 25, 50, 100, 200, 300, and 400 mcg/ml standard solutions were injected and the corresponding peak areas recorded. Figure 10 shows two of the actual chromatograms for the 100 ug/ml and 50 ug/ml standard solutions. The standard calibration curve was prepared daily by plotting the known drug concentrations versus the peak areas recorded (Figure 11).

The dermal dressings containing gentamicin sulfate at two different loading levels (16 and 20 mg) were placed face down, in contact with 20 ml of distilled water contained by the receptor compartment in the Franz cells. Aliquots of 0.5 ml were withdrawn every fifteen minutes for the first three hours and then hourly for the next five hours. Additional aliquots were then extracted at 24, 48 and 72 hours. Ten microliters of each sample was analyzed on the HPLC. The concentrations of the sample drug extracts were then determined from the calibration curve. Dressings were also prepared without the drug. These were extracted, analyzed similarly and used as controls.

Chromatograms of the control samples (blanks) show no peaks throughout the time frame tested (72 hours). Figure 12 shows the chromatogram of a drug sample withdrawn after 5 hours. Other samples also showed this typical pattern. Chromatographic results show the peak areas of the gentamicin sulfate samples to have a linear proportionality to the concentration between 25 and 400 mcg/ml. Drug concentrations below 25 mcg/ml and above 400 mcg/ml were also detectable. However, it was not necessary to investigate outside of this concentration range for the in vitro testing of dressing. Results of the test samples show the amount of drug released increases with time over the investigational period. The gentamicin release follows a typical monolith (matrix type) release kinetics. Results are shown in Figure 13.

Based on these considerations, we constructed Table 5, which shows the elution rates from dual-loaded wound dressings.

PRELIMINARY RESULTS

(20ml Franz Cells, HPLC-grade Water at 37°C)

TIME (HRS)	Drug Delivered mcg/cm²			
	GENTAMICIN		CLINDAMICIN	
	16 mg	20 mg	16 mg	20 mg
1	210	294	NP	138
2	240	366	NP	199
3	316	633	137	359
4	505	724	222	382
5	600	811	226	471
6	699	917	230	549
7	826	1007	NP	NP
8	968	1153	387	668
24	1418	1510	955	1192
48	1575	1778	1119	1401
72	1739	1879	1301	1474

NP = Not Performed

TABLE 5

E. TASK 5. Supply USAIDR with Maximally-Loaded Gentamicin/ Clindamycin Level Dressings.

Both gentamicin and clindamycin are powders, with a potency of approximately 75%; these powders have a maximum concentration that can be incorporated into the liquid oligomer. Once the maximum dual loading has been obtained, any additional drug turns the oligomer/drug mixture into an unworkable slurry which can no longer be evenly spread by means of a knife over roll.

We found experimentally that 17 mg of gentamicin and 17 mg of clindamycin was the maximum amount of the drugs that could be loaded into dual dressings. Ten of these dual loaded dressings were sent to USAIDR for in vivo testing. Concurrently, dressings from the maximally loaded batch, as well as a 10/10 loaded batch were tested in vitro by microbiological zone of inhibition.

1. In Vivo Testing

In vivo evaluation by USAIDR showed significantly reduced bacterial growth over a 72 hour period. Based on the scrub assay technique, nine out of ten dressings completely eliminated the Staphyloccocus aureaus infection, whereas one dressing failed at 10^3 cfu. Utilizing the quantitative biopsy method to measure organisms, six of the dressings completely inhibited growth. Four reduced the bacterial count from 10^7 cfu to 10^5 cfu as seen in the placebos.

2. In Vitro Testing

(a) Methods

While the following tests were not required as part of the contract, they have been included due to their relevance to the ongoing program. Standard solutions of gentamicin, clindamycin and gentimicin/clindamycin combination were prepared and 0.02 ml of each solution was inoculated onto individual sterile filter paper disc. Each disc was then placed on a TSA plate that had been surface inoculated with test organism (each determination performed in duplicate). The plates were then incubated for 18 hours at 30-35°C. The zones of inhibition were measured (mm) and plotted to achieve a log antibiotic activity versus zone size curve.

The patches investigated (5 mm diameter) contained known amounts of clindamycin, gentamicin and gentamicin/clindamycin combinations. The dermal patches were placed on the surface of TSA plates that had been surface inoculated with test organism (each determination performed in duplicate). The plates were then incubated at 30-35°C for 18 hours, and the zones of inhibition were measured (mm).

Negative control discs consisting of patches with no antibiotic were placed onto surface inoculated plates to validate that no antibacterial activity was generated from the patch material alone.

Standard curves were derived against each test organism for gentamicin, clindamycin and a 1:1 gentamicin/clindamycin combination. A strong linear relationship was found between log antibiotic activity and zone size.

Standard curves were used to relate zone sizes of the patches to antibiotic activity of the standard filter paper discs assay (Step #4).

(b) Results

All gentamicin/clindamycin combination patches have antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus.

All clindamycin patches have no activity against Pseudomonas aeruginosa (as expected), but do have antibacterial activity against Staphylococcus aureus.

Antibacterial activity of patches with gentamicin or clindamycin alone can be estimated relative to an equivalent amount of antibiotic placed on a filter paper disc.

The amount of antibacterial activity due to gentamicin in gentamicin/clindamycin patches can be estimated relative to an equivalent amount of gentamicin placed on a filter paper disc, using Pseudomonas aeruginosa as a test organism.

The amount of antibacterial activity due to clindamycin in patches containing gentamicin/clindamycin combinations can not be estimated using the present test organisms.

The results of the in vitro tests performed at MCP Research and Development Center showed the zone of inhibition exhibited by the maximally loaded dressings were significantly larger (Figures 14 and 15). This indicates an increase in antibacterial activity of the maximally loaded dressing compared to those of lower concentrations.

F. PROGRAM SUMMARY

The results obtained thus far with Thermedics' antimicrobial dermal dressings have been very encouraging. The methods required to incorporate gentamicin and clindamycin into the vinyl terminated urethane oligomer have been determined. Analytical methods required to measure the release kinetics of the drugs has been established. Permeation rates of carbon dioxide, oxygen and water through the dressing have been documented, and the advantage of incorporating a small quantity of silicone into the oligomer to reduced adherence to the wound has been demonstrated. Finally, controlled release of the drugs and the ability to significantly reduce microbial growth, both in vitro and in vivo, have been proven.

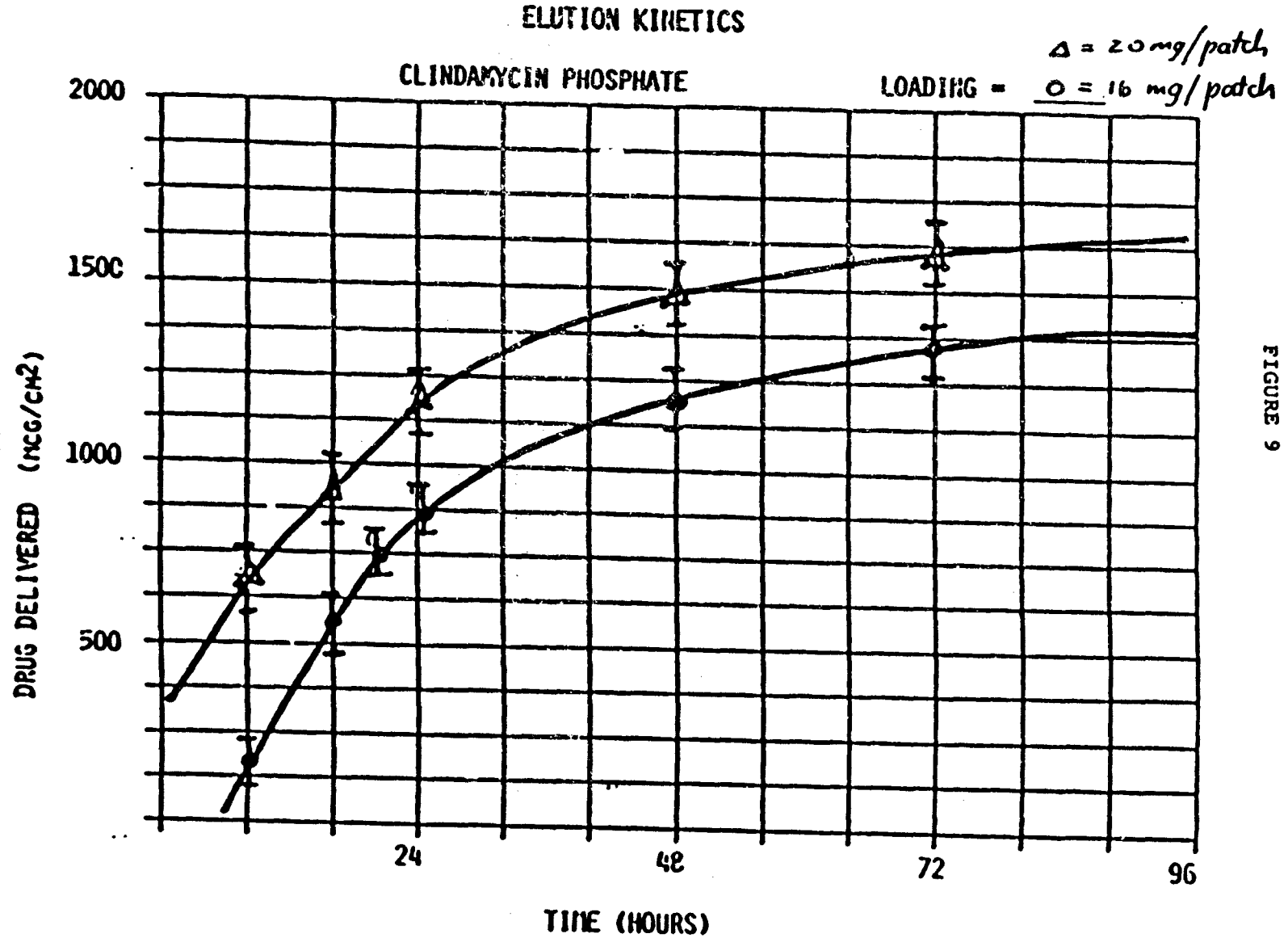
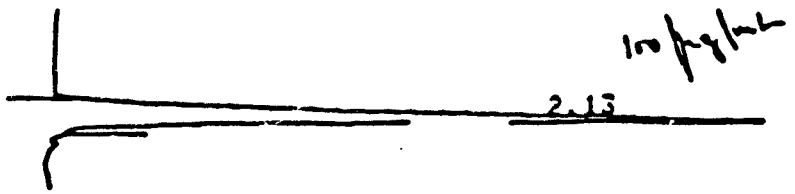

ELUTION KINETICS
CLINDAMYCIN PHOSPHATE
Δ = 20 mg/patch
LOADING = O = 16 mg/patch
2000
1500
1000
500
DRUG DELIVERED (MCG/CM²)
24
48
72
96
TIME (HOURS)

FIGURE 9

NR

INJECT

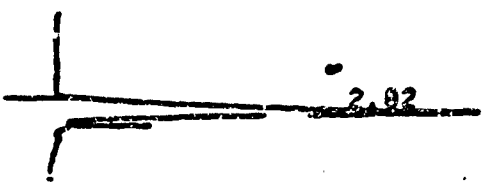


JUL. 6, 1988 03:35:45 CHART 0.75 CM/MIN
RUN #50 CALC #0
COLUMN SOLVENT OPR ID: 6

EXTERNAL STANDARD QUANTITATION

PEAK#	AMOUNT	RT	EXP RT	AREA	RF
	423.84000	2.13		423840 L	0.000000E0
TOTAL	426.84000				

INJECT



JUL. 6, 1988 03:48:48 CHART 0.75 CM/MIN
RUN #51 CALC #0
COLUMN SOLVENT OPR ID: 6

EXTERNAL STANDARD QUANTITATION

PEAK#	AMOUNT	RT	EXP RT	AREA	RF
	211.62000	2.02		211620 L	0.000000E0
TOTAL	211.62000				

Figure 10
Chromatograms of Gentamicin Sulfate
100 mcg/ml and 50 mcg/ml Standards

FIG. 11 STANDARD CALIBRATION CURVE

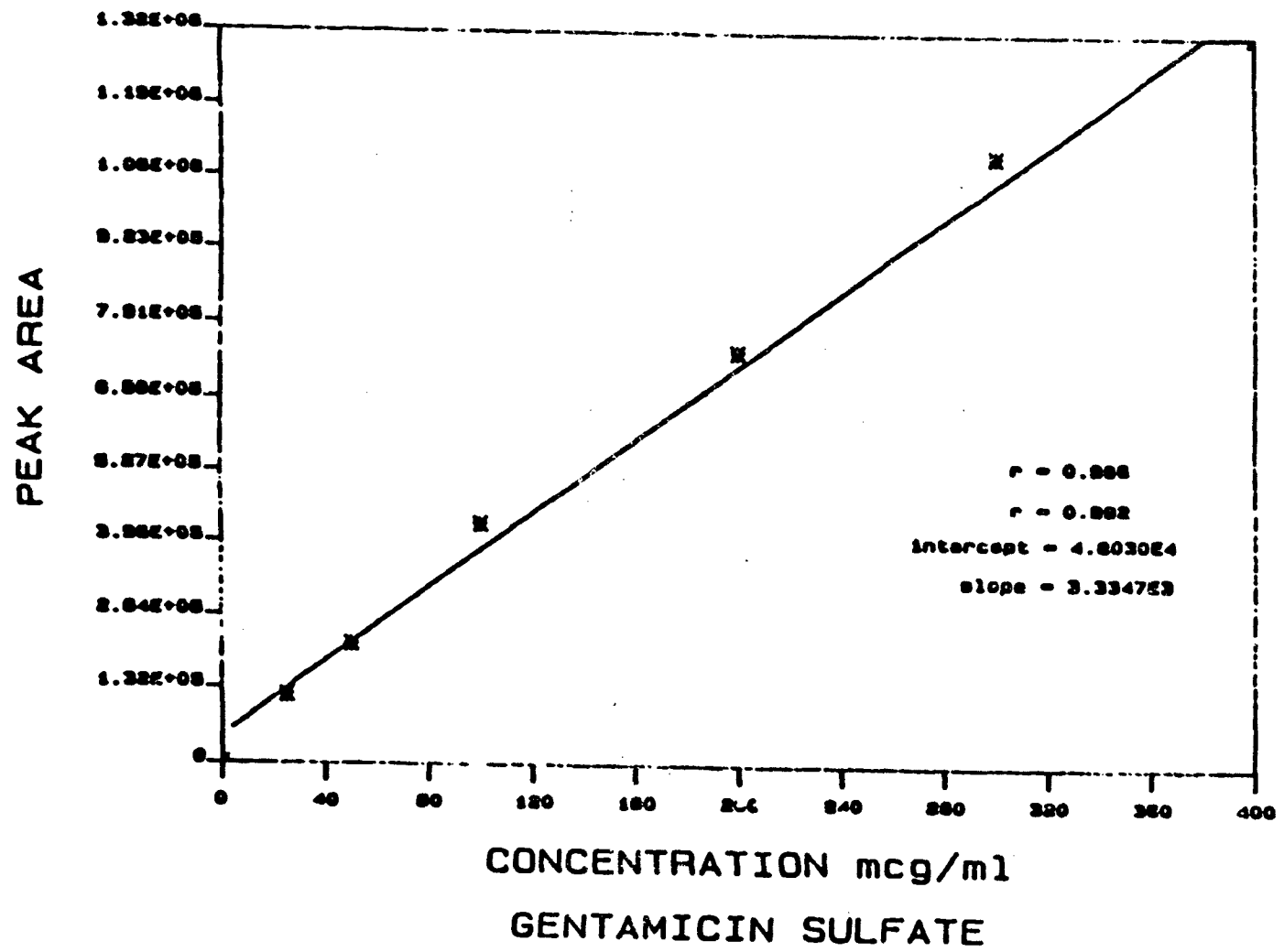
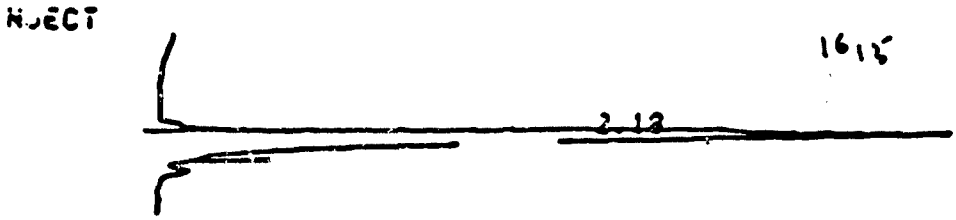


Figure 12
Chromatogram of Sample Extracted
from Franz Cell After Five Hours
for Wound Dressing with 16mg Loading
of Gentamicin Sulfate

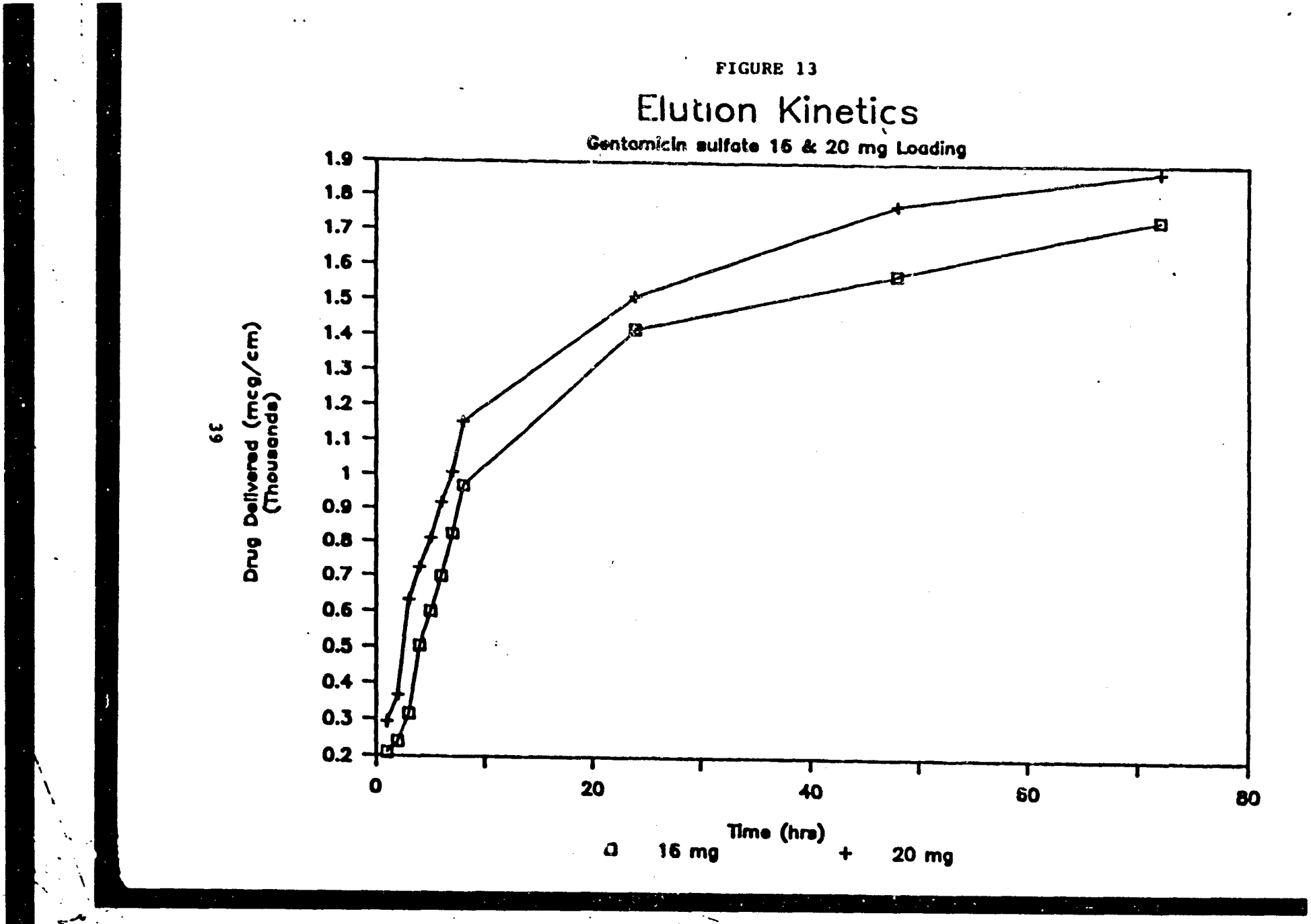


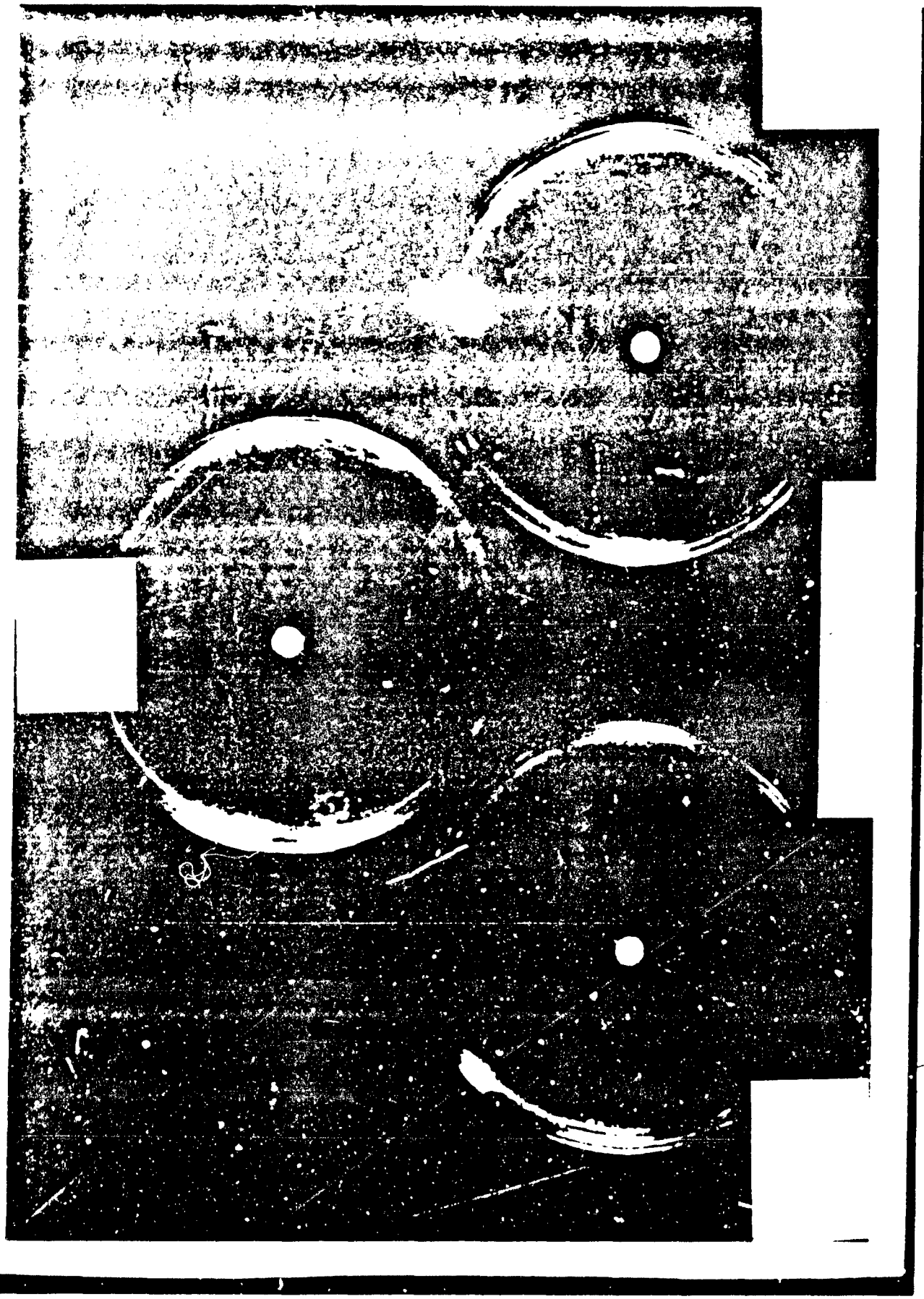
JUL. 6, 1988 11:33:33 CHART 0.75 CM/MIN
RUN 882 CALC 88
COLUMN SOLVENT OPR ID: 6

EXTERNAL STANDARD QUANTITATION

PEAK#	AMOUNT	RT	EXP RT	AREA	RF
	457.98189	2.13		457931 L	0.000000E0
TOTAL	457.98189				

FIGURE 13


Elution Kinetics
Gentamicin sulfate 16 & 20 mg Loading
Drug Delivered (mcg/cm)
(Thousands)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
20
40
60
80
Time (hrs)
16 mg
20 mg



IV. REFERENCES

1. Rovee, D.T., et al., "Effect of Local Wound Environment on Epidermal Healing," Epidermal Healing, Edited by H.I. Marbach and D.T. Rovee, Chicago: Year Book Medi-cal Publishers, Inc., pp. 159-181 (1972).

2. Winter, G.D., "Healing of Skin Wounds and the Influence of Dressings on the Repair Process," Surgical Dressings and Wound Healing, Edited by K.J. Harkiss, Bradford University Press, pp. 46-50.

3. Lobe, T.E., et al., "An Improved Method of Wound Management for Pediatric Patients," J. Ped. Surg., 15:6, 886, (1980).

4. Winter, G.D., "Epidermal Regeneration Studied in the Domestic Pig," Epidermal Wound Healing, edited by Marbach and Rovee, Year Book Medical Publishers, Inc. (1972).

5. Caviak, Y., "The Application of Op-Site to Donor Sites, Burns and Post-Operative Wounds," Aktuelle Traumatologie 10 (1980).

6. McCarthy, D.J., "Polyurethane in the Management of Ulcerating Lesions at the Lower Extremities," J. Am. Pod. Assoc., 73:1 (1983).

7. Conkel, W., "Op-Site Dressing: New Approach to Burn Care," J. Emerg. Nursing (1981).

8. Homsy, C.A., et al., "Materials for Oral Implantation - Biological and Functional Data," JADA 86:817-832 (1973).

9. Weinberg, S., et al., "Response of Oral Tissues to Implants Totally Covered by Mucous Membrane," Oral Sci. Rev., 5:68-84 (1974).

10. Leyden, J.J., Stewart, R., Kligman, A.M., "Updated In Vivo Methods for Evaluation Topical Antimicrobial Agents on Human Skin," J. Invest. Derm., 72:165-170 (1979).

11. Alcid, D.V., and Silijman, S.G., "Simplified Assay for Gentamicin in Presence of Other Antibiotics," Antimicrog. Agents Chemother., 3. 559 (1973).

REFERENCES (con't.)

12. Holmes, R.K., and Sanford, J.P., "Enzymatic Assay for Gentamicin and Related Aminoglycoside Antibiotics," J. Infect. Dis., 129, 519 (1974).

13. Mahon, W.A., Feldman, R.I. and Scherr, G.H., "Hemagglutination Inhibition Assay for Gentamicin," Antimicrog. Agents Chemother., 11, 359 (1977).

14. Mahon, W.A., Ezer, J., and Wilson, T.W., "Radio Immuno Assay for Measurement of Gentamicin in Blood," Antimicrog. Agents Chemother., 3, 585 (1973).

15. Mitra, S.K., Yoshikawa, T.T., Hansen, J.L., Nisson-Ehle, I., Palin, W.J., Shot.., M.C. and Guze, L.B., "Serum Gentamicin Assay by High Performance Liquid Chromatography," Clir, Chem. 23/12, 2275 (1977).

16. Peng, G.w., Gadalla, M.A.F., Peng, A., Smith, V., and Chiou, W.L., "High Performance Liquid Chromatographic Method for Determination of Gentamicin in Plasma," Clin. Chem., 23/10, 1938 (1977).